another must obviously have been greatly facilitated by their possession of wings, may in theory appear plausible enough, but when tested on the actual distribution of the species and subspecies it proves to be of much less importance than commonly supposed; it rests, in reality, on the confusion of two different things: the power of flight no doubt would *enable* a bat to spread over a much larger area than non-flying mammalia, but as a matter of fact, only in a very few cases is there any reason to believe that it has *caused* it to do so.[7] . . . A few of the more striking examples may be mentioned here: a species of *Pteropus* inhabits the island of Pemba, south of Zanzibar, but although the island is separated from Africa by a channel only 35–40 miles wide, not this particular species only, but the whole genus is unknown from any part of the adjacent continent;[8] although absent from Africa the genus *Pteropus* is distributed all over the Malagasy region,[9] and each group of islands . . . has its own peculiar species, intermigration between the groups of islands is unknown; the Epomophorine section of fruit-bats is distributed over the whole of the Ethiopian region (eight genera, nineteen forms), but not a single form has spread to any island of the Malagasy region; the *Pteropus melanotus* group of [five] species is distributed over the Andamans, Nicobars, Nias, Engano and Christmas Island (south of Java), and the whole group is confined to this chain of islands, no form having spread to the neighboring Malay Peninsula or Sumatra. . . . The fruit-bat faunas of the Malay Peninsula, Sumatra and Borneo are closely interrelated, like their mammalian faunas in general, but each has some distinct autochthonous forms of fruit-bats (Borneo even two autochthonous [?][10] genera), as it has of other Mammalia; the Javan mammalian fauna in general is more peculiar, both by the absence of some of the forms found in Sumatra and the Malay Peninsula, and by the greater percentage of autochthonous forms, and this is again borne out by the Megachiropterine fauna of the island . . .; the *Pteropus rayneri* group is represented probably all over the Solomon Islands, but it has differentiated into five distinct species, one in the Bougainville group, a second on Villa Lavella, a third in the New Georgia group, a fourth on Guadalcanar and a fifth on San Cristobal. This . . . tends to show that the present distribution of the Megachiroptera has not been influenced to any great, and as a rule not even to any appreciable, extent by their power of flight; if it had, the fruit-bat fauna of one group of islands could not, so commonly as is actually the case, differ from that of a neighboring group or continent, and the tendency to differentiation of insular species or forms would have been neutralized by the free intercourse between neighboring faunas.

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[7] The preponderance of bats over the characteristic Indo-Malayan non-volant types in the fauna of the Andaman and Nicobar Islands appears to be an instance in which wings have played a part in distribution (Miller, *Proc. U. S. Nat. Mus.*, XXIV., pp. 790–791, May 28, 1902); the presence of a slightly modified species of *Nycteris*, a characteristic American type of bat, as the only indigenous mammal of the Hawaiian Islands is probably another case of the same kind.

[8] Conversely, six species of European bats (*Myotis myotis, M. dasycneme, M. emarginatus, Pipistrellus natusii, Eptesicus nilssonii* and *Vespertilio murinus*, although occurring on or near the west coast from Brittany northward, are not known to have become established in England.

[9] It ranges eastward "through the . . . Oriental and Australian regions to the Samoa Islands."

[10] Further exploration will probably show that both occur on the peninsula or in Sumatra.

SPECIAL ARTICLES

THE PRODUCTION OF SPERM ISO-AGGLUTININS BY OVA

I. If one allows unfertilized eggs of *Arbacia* to stand in a quantity of sea-water that does not exceed about ten times the volume of the eggs, the sea-water soon becomes perceptibly tinged with the red coloring matter of the eggs. If now a few drops of such supernatant sea-water be added to 2 or 3 c.c. of a milky suspension of active sperm of the same species, a strong agglutination of the sperms immediately ensues, producing sperm-masses easily visible to the naked eye. In the course of three to five minutes reversal takes place, the masses become converted into their constituent cells, and considerable activity may be observed after the reversal on microscopical examination. The substance which produces this phenomenon may be called a sperm agglutinin, and since it is produced by the same species, an iso-agglutinin.

This basic phenomenon was studied in three

ways: (1) In a test tube or vial containing 2 or 3 c.c. of a milky sperm suspension, as above described. (2) Drops of the sperm suspension and of the egg-extract may be placed side by side on a slide and connected under the microscope, so as to observe the inception and progress of the phenomenon. (3) The best method of studying the detail of the phenomenon is to mount some of the sperm suspension beneath a long cover glass supported by glass rods 0.5 to 1 mm. in diameter, and to inject a drop of the egg-extract with a capillary pipette into the suspension. If this be observed under a low power of the microscope, one sees that the drop fills with sperm exhibiting increase of activity, which immediately agglutinate into small masses, which then unite with great rapidity to form larger masses spaced throughout the drop. At the margin of the drop and the sperm suspension a ring of agglutinated sperms forms which ruptures in numerous places, each segment contracting to form a mass, so that the continuous ring becomes a chain of beads visible to the naked eye. The ring forms in a second or two and breaks into masses in two or three seconds. The agglutination is totally reversible, so far as the visible effect is concerned, and the freed spermatozoa, or some of them, appear to regain full activity; moreover, the spermatozoa between the masses are in active movement throughout.

The reversal of the reaction consists in the resolution of the agglutinated masses into their individual cells, but this does not mean a return to their original physiological condition; for if the agglutinating solution (egg-extract) be sufficiently strong, after a period of intense stimulation followed by agglutination and reversal, the movements of the spermatozoa gradually cease entirely and in ten minutes they are no longer capable of fertilizing ova dropped in with them.

The agglutination is a factor of (1) the activity and relative density of the spermatozoa in the suspension, and (2) of the strength of the agglutinating solution. The more active the sperms and the greater the density of the suspension (within certain limits) the more rapid is the onset of the agglutination and the larger the agglutinated masses. The agglutinating medium may of course be made to vary in strength, and hence in agglutinating power by various means, *e. g.*, by crushing ovaries and eggs in about their own bulk of sea-water, or extracting in distilled water, very powerful solutions may be made.

The agglutinating agent is not readily destroyed by heat. After boiling and maintaining at a temperature of 95° to 98° C. for about 70 minutes, the agglutinating action of a strong extract was very much reduced but was not entirely lost.

No other tissue of the sea-urchin, so far as observed, produces an agglutinating agent for the sperm of the species: (1) The serum from the body-cavity, whether of males or females, is entirely neutral and the spermatozoa swim in it normally. But the serum forms a powerful agent for extracting the agglutinin from the eggs after the latter are removed from the ovary, though in the intact animal in which the eggs are separated from the serum by the ovarian membrane no extraction of agglutinin takes place. (2) Large pieces of the intestine were cut up in sea-water or in distilled water, the latter especially extracting colored matters in large quantity; but no sperm agglutinin could be detected in the filtrate.

The agglutination reaction fixes the agglutinin, as is readily proved by the fact that it disappears from an agglutinated sperm suspension, if not present in excess. Whereas an equal dilution with sea-water of the original egg-extract is still highly agglutinative. The agglutinative substance, therefore, presumably enters into combination with some sperm substance acting in this respect similarly to its antigen.

II. The results are essentially the same for *Nereis,* except that the visible agglutination is not reversible to the same extent, a difference which is probably of purely secondary significance. Moreover, the agglutinin is produced by the eggs in sea-water only in small quantities before fertilization, but in very large quantities at the moment of fertiliza-

tion. The eggs of *Nereis* when obtained are in the stage of the germinal vesicle, whereas those of *Arbacia* have formed both polar bodies, and this is probably correlated with the different rates of secretion of the agglutinin. The agglutinin of *Nereis* is moreover much more thermolabile, being destroyed at 95° C. in ten minutes. In *Nereis* also it is difficult to test other tissues satisfactorily, as the animal is practically a bag of eggs when sexually mature, but such tests as were made indicate that the eggs alone secrete the agglutinin. In other respects *Nereis* is a more favorable form for study, as the sensitiveness of the spermatozoa is unsurpassed.

III. As regards the important question of specificity, it seems very probable *a priori* that a substance which is produced only by the eggs of a given species, and which is agglutinative for the sperm of that species should be specific. Now the egg-extract of *Nereis,* which agglutinates *Nereis* sperm, is entirely devoid of agglutinating effect on *Arbacia* sperm, so that there appears to be specificity of the *Nereis* agglutinin to this extent at least. But the egg-extract of *Arbacia,* on the other hand, is extremely toxic and agglutinative for *Nereis* sperm, so that in this case either the agglutinin from *Arbacia* eggs is not specific for its own sperm, or the egg-extract contains, in addition to the specific agglutinin, another toxic substance. The latter alternative is probable, as is shown by the fact that the serum of the cœlomic fluid of *Arbacia,* which is not in the least toxic or agglutinative for *Arbacia* sperm, is as strong an agglutinative agent for *Nereis* sperm as the egg-extract of *Arbacia.* The presence of at least two sperm agglutinating substances in *Arbacia* is therefore certain, viz., one in the egg-extract, which is agglutinative for *Arbacia* sperm, and one in the cœlomic fluid not agglutinative for *Arbacia* sperm but agglutinative for *Nereis* sperm. I assume, therefore, provisionally, that both are present in the egg-extract, and that the sperm iso-agglutinin of *Arbacia* eggs is probably specific.

This assumption is rendered more probable by an observation made only once, and at the end of the season, so that its repetition this year is impossible. I had preserved a strong egg-extract of *Arbacia* for seventeen days. This had been tested at various times with *Arbacia* sperm and found to retain its agglutinating power without any observable diminution. But on the seventeenth day, when the last *Nereis* of the season was brought in, I was surprised to find that the egg-extract in question had lost its agglutinating power on the *Nereis* sperm. The same sperm was agglutinable with an egg-extract of *Nereis* eight days old; so that the difficulty could not be with the sperm. And the same egg-extract of *Arbacia,* on retesting, was found to retain its agglutinating power on the *Arbacia* sperm apparently unaltered. The specific agglutinin is therefore relatively stable with reference to the conditions involved, and the non-specific agglutinin or toxin is relatively labile. It might, therefore, be possible to separate them also by heat or other means.

One of these would be to fix the non-specific agglutinin of *Arbacia* egg-extract with *Nereis* sperm, and test the filtrate for the persistence of the assumed specific agglutinin with *Arbacia* sperm. If the *Arbacia* agglutinin were found to persist after the filtrate had lost its *Nereis* agglutinating power, the presence of the two agents in the egg-extract would be demonstrated. Or if, after completely fixing the specific agglutinin of *Arbacia* egg-extract with *Arbacia* sperm, a *Nereis* agglutinating agent were still found, the same conclusion would be inevitable. Unfortunately, by the time this stage of the analysis was reached, the season for both forms was over, and this experiment must be postponed for a year.

IV. The egg-extracts contain not only an agglutinin for the spermatozoa, but also an aggregative agent, *i. e.,* a substance towards which the spermatozoa are positively chemotactic. This may be readily demonstrated by the form of the reaction when a drop of the fluid to be tested is injected into a sperm suspension beneath a raised cover glass. If an aggregative agent be present, a ring of spermatozoa forms at or within the margin of the

drop, depending on the strength of the agent, and a clear zone arises between this ring and the general sperm suspension. The clear zone is produced by migration of spermatozoa to the ring; in case the agent is very strong the ring expands, owing to immigration of spermatozoa, but the clear zone is never obliterated, no matter how much the ring may expand. In the case of *Nereis,* which has unusually large spermatozoa, the passage of spermatozoa across the clear zone to the ring may be readily studied under a low power of the microscope, and it gives the impression of a regular rain falling on the ring.

In the case of *Nereis* all acids tested are aggregating agents (CO_2, H_2SO_4, HNO_3, HCl and $CH_3 \cdot COOH$ were studied), but do not agglutinate, and alkalis (K, OH and NaOH only studied) are agglutinative but not aggregative. The sperm of *Nereis* is extremely sensitive to acids, reacting positively to *N*/1,000 H_2SO_4, HCl, HNO_3 and *N*/2,000 acetic, and to 1/200 dilution of a saturated solution of CO_2 in sea-water. The phenomena of aggregation were therefore studied, particularly in the case of *Nereis.* The sensitiveness of *Arbacia* sperm to acids is three or four times less than that of *Nereis* sperm, but the reactions are in the same sense.

If an acid or other aggregative agent alone be present in the drop tested in the suspension beneath a raised cover slip, the ring which forms is perfectly continuous and the individual spermatozoa are separate. If an agglutinin as well as an aggregative agent be present, the ring forms and breaks up into agglutinated masses. If the agglutinin alone be present no ring proper forms, or there is no outer clear zone, and agglutinated masses form within the drop as described. The egg-secretions give the double reaction.

V. Von Dungern's experiments (*Zeitsch. f. allgem. Physiologie,* I., pp. 34–55, 1902) are the only ones, so far as I know, in which the production of sperm agglutinins by ova was investigated, and he discovered only hetero-agglutinins, no iso-agglutinins. He did, indeed, describe the loss of motility of spermatozoa in egg-extracts of the same species, but he entirely missed the phenomenon of agglutination and its reversal. He reveals the reason for this failure by his remark that he always examined for the effect of the "egg-poison" about half an hour after its addition to the sperm; but the phenomenon of agglutination and its reversal are completed in about five minutes.

Von Dungern also made experiments on the production of immune sera by injection of ova and spermatozoa separately into rabbits, and found that both caused the production of a sperm agglutinin in the rabbit's serum. From this he concludes that both kinds of reproduction elements possess chemically identical complexes of molecules in the protoplasm. While this may be admitted as at least a very probable conclusion, his farther conclusion that fertilization does not depend upon any specific antagonism between ovum and sperm, but is conditioned by the similarity of their protoplasms, is not well founded, for the egg is a very complicated chemical system, and it certainly contains molecules antagonistic to sperm, even if, as von Dungern's experiments indicate, it also contains some that are not.

VI. The existence of sperm iso-agglutinins in ova offers the possibility of an explanation of the specificity of fertilization on the basis of the laws governing antigens and antibodies, if these agglutinins are specific, as is so strongly suggested by the experiments. The union of ovum and spermatozoon is not a process in which the sperm penetrates by virtue of its mechanical properties, but one in which a peculiarly intimate and specific biochemical reaction plays the chief rôle. A later publication will give the details of the experiments and a more complete analysis of the behavior of the spermatozoa in fertilization.

Frank R. Lillie

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Preliminary Note on Pristina and Naidium

These two genera, *Pristina* and *Naidium,* of the aquatic oligochætes, have been combined and separated by recent systematists with quite startling rapidity. The former genus was first described by Ehrenberg in